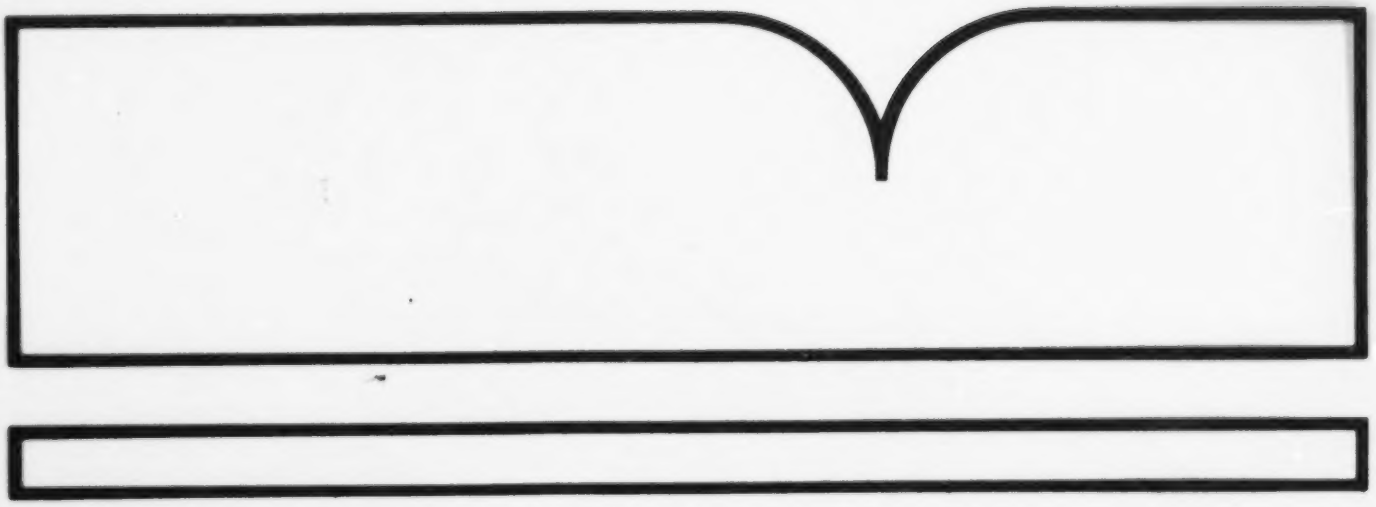
PB82-249343

Microbial Aspects of Groundwater Pollution

(U.S.) Robert S. Kerr Environmental Research Lab., Ada, OK

Jul 80



U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. R
EPA 600/J-81-574		PB82-249343

4. TITLE AND SUBTITLE	5. REPORT DATE
MICROBIAL ASPECTS OF GROUNDWATER POLLUTION	July 1980
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
C. P. Gerba and J. F. McNabb	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Robert S. Kerr Environmental Research Lab. - Ada, OK Office of Research and Development U.S. Environmental Protection Agency Ada, Oklahoma 74820	13. TYPE OF REPORT AND PERIOD COVERED
	14. SPONSORING AGENCY CODE EPA/600/15

15. SUPPLEMENTARY NOTES

ASM News Vol, 47, No. 8, 1981 326-329

16. ABSTRACT

Microbiology, as it relates to ground-water pollution, can be divided into two distinct areas of interest: the fate and transport of pathogens, and the microbial ecology of subsurface regions and the potential interactions of microorganisms with chemical or microbial pollutants. This article discusses the general state-of-knowledge in these areas and presents some of the difficulties in studying subsurface microbial ecology. Determinations of the nature and extent of microbial activity in the subsurface, the movement and fate of pathogens, and the controlling environmental factors are essential if ground-water quality is protected.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Ground Water	Virus adsorption	57-H
Bacteria	Virus survival	57-K
Virus	Ground-water sampling	57-U
Indicator Organisms		44-G
Microorganisms		68-D
Pollution		
Degradation		

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to the public.	Unclassified.	
	20. SECURITY CLASS *(This page)*	22. PRICE
	Unclassified.	

EPA Form 2220-1 (9-73)

EPA 600/J-81-574

FEATURE ARTICLES

Microbial Aspects of Groundwater Pollution

C. P. GERBA[1] and J. F. McNABB[2]

Department of Microbiology, University of Arizona, Tucson, Arizona 85721,[1] and Robert S. Kerr Environmental Research Laboratory, U.S. Environmental Protection Agency, Ada, Oklahoma 74820[2]

Although groundwater has not received as much consideration as surface water in the past, recent events, such as those at Love Canal, have focused attention on this valuable national resource. Groundwater—water beneath the land's surface—constitutes 95% of the nation's freshwater reserves. Approximately 50% of the total population of the United States, including 94% of the rural population, depends on groundwater as a source of drinking water, and these percentages are increasing (4).

Groundwater has historically been considered a reliable and safe source of water, protected from surface contamination by the upper soil mantle which has been thought to be a "living filter," removing pollutants as the water percolates downward through the soil. However, a number of well-documented outbreaks of hepatitis A and viral gastroenteritis traced to contaminated groundwater, as well as numerous reports of chemical contamination, have destroyed the widely held misconception that groundwater is safe from pollution and have necessitated a reassessment of what is known about the fates of pathogenic microorganisms and chemical pollutants in soil and groundwater.

Microbiology as it relates to groundwater pollution can be divided into two distinct areas of interest: the first area concerns the fate and transport of pathogens, and the second area deals with the microbial ecology of subsurface regions and the potential interactions of microorganisms with chemical or microbial pollutants.

Fate and Transport of Pathogens in Groundwater

The fate of pathogenic bacteria in soil has been studied since the early part of this century as a result of disease outbreaks associated with the contamination of sewage-irrigated food crops. However, it has only been in the last decade that a serious effort has been made to understand the fate and migration of human enteric viruses in the subsurface environment. Viruses are known to enter groundwater under certain conditions, but these conditions have not been adequately defined. The effectiveness of removal of viruses and bacteria during the percolation of wastewater through the soil is dependent upon the retention of the particles by the soil matrix and the ability of the organisms to survive. The longer the organisms survive, the greater the chance that an event will occur that will promote their penetration into groundwater.

Removal of bacteria by soil is due to both filtration and adsorption, whereas removal of viruses is dependent entirely on adsorption (5). Understanding the physicochemical properties of soil as related to virus adsorption has been seen as an aid in understanding the potential for groundwater contamination. Virus adsorption to soil surfaces is believed to be largely governed by electrostatic double-layer interactions and van der Waals forces (5). Thus, the surface charges on both the virus and the soil and factors controlling the net charge are believed to be important in determining the efficiency of virus adsorption.

Three factors would appear to have a large influence on both the survival and the vertical migration of microorganisms through soil. These are (i) the climate, (ii) the nature of the soil, and (iii) the nature of the microorganisms.

Temperature and rainfall are two climatic factors that may determine viral and bacterial survival. The survival of microorganisms is greatly prolonged at low temperatures; below 4°C they can survive for months or even years. At higher temperatures, inactivation or die-off is fairly rapid. A principal factor determining the survival of bacteria and viruses is loss of moisture during drying of the soil (5, 20; C. J. Hurst, J. C. Lance, and C. P. Gerba, manuscript in preparation).

The nature of the soil also plays a major role in determining survival. Soil properties influence moisture-holding capacity, pH, and organic matter content, all of which control the survival of bacteria in soil. Longer survival times of bacteria have been observed in alkaline than in acid soils (pH 3 to 5) and when large amounts of organic matter are present (5).

Virus survival in a particular soil appears to be directly related to the degree to which the virus

adsorbs (C. J. Hurst, C. P. Gerba, and I. Cech, manuscript in preparation). Although the mechanism of this protective effect has yet to be investigated, the mechanisms of virus inactivation have recently been reported. Yeager and O'Brien (21) suggest that polioviruses are inactivated by different mechanisms in moist and drying soils. Inactivation under either condition includes disassociation of the viral genomes and capsids and subsequent degradation. In the case of moist soils, however, the viral ribonucleic acid is damaged before release from the viral capsid. The nature of the microorganism will also play a role in survival. Resistance of microorganisms to environmental factors will vary among different species as well as among strains.

Soil microorganisms may play a role in virus survival (Hurst, Lance, and Gerba, in preparation; Hurst, Gerba, and Cech, in preparation). In a comparative study of the survival of enteric viruses in soil under aerobic and anaerobic conditions, it was found that virus survival was much longer under anaerobic than under aerobic conditions. The results indicated that aerobic, but not anaerobic, soil microorganisms are antagonistic to virus survival. If so, this would be important, since most groundwaters are assumed to be anaerobic.

Under the proper conditions bacteria and viruses are known to travel great distances underground. Human enteric viruses have been shown to travel as far as 408 m horizontally in groundwater (18). Coliphages and coliform bacteria have been observed to travel 900 m in groundwater from the site of application (15, 16). With most soils, it appears that bacteria and viruses are largely retained near the soil surface, but it has not yet been possible to establish firm predictive correlations between retention and soil factors. The surface area, cation exchange capacity, organic matter content, and pH of the soil appear to be the most significant for virus removal.

Establishment of predictive relationships between soil factors and virus adsorption is further complicated by genetic variability among the different types and strains of viruses. In recent studies the efficiency of virus adsorption was found to be dependent not only on the type of virus but also on the particular strain (7). Differences in adsorption among different strains of the same virus probably result from variability in the configuration of proteins on the outer capsid of the virus, since this will influence the net charge of the virus. The net charge of the virus would affect the electrostatic potential between virus and soil and thereby influence the degree of interaction between the two particles.

Unfortunately, viruses cannot be considered permanently immobilized on soil particles. This became clear in a study of a wastewater land application site in Florida by Wellings et al. (19). Viruses remained undetected in wells below the soil surface until after periods of heavy rainfall which resulted in the downward migration of viruses from the surface. Subsequent laboratory studies confirmed that viruses previously adsorbed near the soil surface desorb and migrate further through the soil column (10). The degree of elution that occurs during a rainfall event also appears to be dependent on both the type and the specific strain of virus (11). Virus eluted near the soil surface will eventually readsorb further down a soil column (10), but it has been speculated that viruses could travel vertically through the soil by a chromatographic effect controlled by periodic rainfall.

From the foregoing discussion it is apparent that many factors control the removal of pathogenic bacteria and viruses during percolation of sewage through soil. Most of this article has dealt with the fate of viruses because of their apparent greater potential for health problems associated with land disposal of wastes. It is certain that in many instances bacteria are not adequate indicators of viral pollution. Furthermore, it is questionable whether bacterial standards for surface water will be adequate for groundwater, especially since groundwater is often used without any treatment and has been responsible for a great portion of the reported outbreaks of waterborne diseases. New bacterial and viral standards should be developed to control groundwater pollution.

Microbial Activity in Groundwater Environments

A great deal is known about the physical nature of geological formations containing groundwater, but relatively little is known concerning the occurrence of indigenous microorganisms, the extent and nature of any microbial activity, and the microbial activity that might occur after the introduction of chemical or microbial pollutants. What is known has been learned primarily by soil and petroleum microbiologists working with surface soils or deep petroleum-bearing formations. The water-bearing regions between the surface and deep geological formations remain relatively unstudied.

As a rule microbial ecologists in the past have had insufficient justification to warrant the effort and expense to study freshwater aquifers to any extent. Today there is a growing recognition by both laymen and scientists of the importance of groundwater as a natural resource, and we are rapidly awakening to the fact that groundwater is not safe from pollution, but rather is severely

threatened by a myriad of industrial and domestic waste sources. Determining the nature and extent of microbial activity in aquifers and in the overlying unsaturated zones has become essential for predicting the ultimate fates of pollutants placed in soil and groundwater, inasmuch as biodegradation is apparently the only significant process that can transform organic pollutants after they enter such systems (13). However, there are numerous problems involved in determining the biodegradative potentials of subsurface environments.

The problems and expense involved in obtaining representative and uncontaminated samples from surbsurface environments are the main deterrents to subsurface investigations. Obviously, terrestrial environments far below the earth's surface cannot be directly examined. Instead, biological activities in subsurface environments must be studied by examining natural springwaters or by drilling wells and examining well waters and cores. Springwaters give an indication of the chemical nature of the producing aquifer, but they are not necessarily biologically or chemically representative of water within the aquifer. In essence, they provide information concerning the biological situation in the vicinity of the outcrop where the spring occurs. Well waters are considered to provide more representative information concerning subsurface ecosystems than that obtained from springwaters, but wells are easily contaminated during drilling and sampling operations. Also, the organisms found in well waters may not be representative of the microbial population in an aquifer, but rather may represent only the population in the immediate vicinity of the well. Studies with surface soils indicate that the great majority of soil organisms are firmly attached to soil particles (1), and, therefore, organisms native to an aquifer may not be present in well water during pumping. Samples obtained aseptically from undisturbed cores undoubtedly provide the most representative information concerning subsurface microbial activity, but the acquisition of such samples is difficult. In addition, subsurface strata are not homogeneous, and cores or water samples from a single site or depth may not be representative of the entire formation. However, the difficulties in attempting to obtain representative and uncontaminated samples are not insurmountable if reasonable precautions are taken (M. R. Scalf, J. S. Fryberger, J. F. McNabb, W. J. Dunlap, and R. L. Crosby, *Manual of Ground-Water Quality Sampling Procedures*, in press).

Once a sample is obtained, analytical methodology is another problem, as it is in the study of any natural ecosystem. In the past, most investigators used cultural techniques that were selective for specific microorganisms and, therefore, may have missed the presence of others in the same sample.

Despite these difficulties, there is sufficient evidence that diverse microbial populations exist in many subsurface regions, including aquifers (2, 9, 14). Numerous investigators have found sulfate reducers, methanogens, and heterotrophs in well waters and cores, and a good case can be made that these isolated organisms were metabolically active in their natural habitat (3, 6). Several studies involving the direct recharge of wastes into aquifers also provide strong circumstantial evidence that biodegradation of some organic pollutants occurs in groundwater (12, 17). However, there is currently very little quantitative information on microbial activity in subsurface regions, especially after the introduction of pollutants. We hope that biochemical techniques for determining biomass, metabolic activity, degradative potential, and the community structure of surface materials will be applicable to subsurface materials, although subsurface samples may have lower levels of biological activity.

Information on the kinetics of biodegradation is needed for mathematical models that are being developed to predict the movement and fate of pollutants in groundwater. Correlations of (i) environmental conditions controlling microbial activity, such as oxidation-reduction potentials, (ii) the presence of microorganisms, and (iii) the kinetics of degradation are needed for modeling purposes and for use in manipulating subsurface microbial activity to clean up aquifers that are already polluted. Rehabilitation of aquifers contaminated by gasoline has already been shown to be feasible in some cases (8). The possibility that microbial processes could be managed in other cases to protect or clean up groundwater is only conjecture at this time, but it should not be dismissed until more is known.

Conclusion

Less is known about the microbiology of groundwater than about any other part of the biosphere. The significance of microbiology as it relates to groundwater resides in the interactions of pollutants and microorganisms in the subsurface and the effects of such interactions on the quality of groundwater. Determinations of the nature and extent of microbial activity in the subsurface, the movement and fate of pathogens, and the controlling environmental factors are essential if groundwater quality is to be protected.

Literature Cited

1. **Balkwill, D. L., T. E. Ruckinsky, and L. E. Casida.** 1977. Release of microorganisms from soil with

respect to transmission electron microscopy viewing and plate counts. Antonie van Leeuwenhoek J. Microbiol. Serol. **43**:73–87.

2. **Davis, J. B.** 1967. Petroleum microbiology. Elsevier-North Holland Publishing Co., New York.
3. **Dockins, W.S., G. J. Olson, G.A. McFeters, and S. C. Turbak.** 1980. Dissimilatory bacterial sulfate reduction in Montana groundwaters. Geomicrobiol. J. **2**:83–98.
4. **Freeze R. A., and J. A. Cherry.** 1979. Groundwater. Prentice-Hall, Inc., Englewood Cliffs, N.J.
5. **Gerba, C. P., C. Wallis, and J. L. Melnick.** 1975. Fate of wastewater bacteria and viruses in soil. Proc. Am. Soc. Civ. Eng. **181**:157–174.
6. **Godsy, E. M., and G. G. Ehrlich.** 1978. Reconnaissance for microbial activity in the Magothy Aquifer, Bay Park, New York, four years after artificial recharge. J. Res. U.S. Geol. Surv. **6**:829–836.
7. **Goyal, S. M., and C. P. Gerba.** 1979. Comparative adsorption of human enteroviruses, simian rotavirus, and selected bacteriophages to soils. Appl. Environ. Microbiol. **38**:241–247.
8. **Jamison, V. W., R. L. Raymond, and J. O. Hudson, Jr.** 1975. Biodegradation of high-octane gasoline in groundwater. Dev. Ind. Microbiol. **16**: 305–312.
9. **Kuznetsov, S. I., M. V. Ivanov, and N. N. Lyalikova.** 1963. Introduction to geological mirobiology. McGraw-Hill Book Co. New York.
10. **Lance, J. C., C.P. Gerba, and J. L. Melnick.** 1976. Virus movement in soil columns flooded with secondary sewage effluent. Appl. Environ. Microbiol. **32**:520–526.
11. **Landry, E. F., J. M. Vaughn, M. Z. Thomas, and C. A. Beckwith.** 1979. Adsorption of enteroviruses to soil cores and their subsequent elution by artificial rainwater. Appl. Environ. Microbiol. **38**:680–687.
12. **Leenheer, J. A., R. L. Malcomb, and W. R. White.** 1976. Investigation of the reactivity and fate of certain organic components of an industrial waste after deep-well injection. Environ. Sci. Technol. **10**:445–451.
13. **McCarty, P. L., B. E. Rittman, and M. Reinhard.** 1980. Processes affecting the movement and fate of trace organics in the subsurface environment, p. 93–117. *In* T. Asano and P. V. Roberts (ed.), Wastewater reuse for groundwater recharge. Office of Water Recycling, California State Water Resources Control Board, Sacramento.
14. **McNabb, J. F., and W. J. Dunlap.** 1975. Subsurface biological activity in relation to ground-water pollution. Ground Water **13**:33-44.
15. **Noonan, M. J., and G. N. Martin.** 1977. Effects of domestic wastewater disposal by land irrigation on groundwater quality at the central Canterbury plains. Water and soil technology, publication no. 7. Ministry of Works and Development, Christchurch, New Zealand.
16. **Noonan, M. J., and J. F. McNabb.** 1979. Contamination of Canterbury groundwater by viruses. *In* M. J. Noonan (ed.), The quality and movement of ground water in alluvial aquifers of New Zealand. Technical bulletin no. 2. Lincoln College, Canterbury, New Zealand.
17. **Roberts, P. V., P. L. McCarty, M. Reinhard, and J. Schreiner.** 1980. Organic contaminant behaviour during ground water recharge. J. Water Pollut. Control Fed. **52**:161–172.
18. **Vaughn, J. M., and E. F. Landry.** 1977. Nassau-Suffolk regional planning board virus study. Hauppauge, New York.
19. **Wellings, F. M., A. L. Lewis, and C. W. Mountain.** 1974. Virus survival following wastewater spray irrigation of sandy soils, p. 253–260. *In* J. F. Molina and B. P. Sagik (ed.), Virus survival in water and wastewater systems. Center for Research in Water Resources, Austin, Tex.
20. **Yeager, J. G., and R. T. O'Brien.** 1979. Enterovirus inactivation in soil. Appl. Environ. Microbiol. **38**:694–701.
21. **Yeager, J. G., and R. T. O'Brien.** 1979. Structural changes associated with poliovirus inactivation in soil. Appl. Environ. Microbiol. **38**:702–709.